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ENZYME TECHNOLOGY FOR SHIPBOARD WASTE MANAGEMENT

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ENZYME TECHNOLOGY FOR SHIPBOARD WASTE MANAGEMENT

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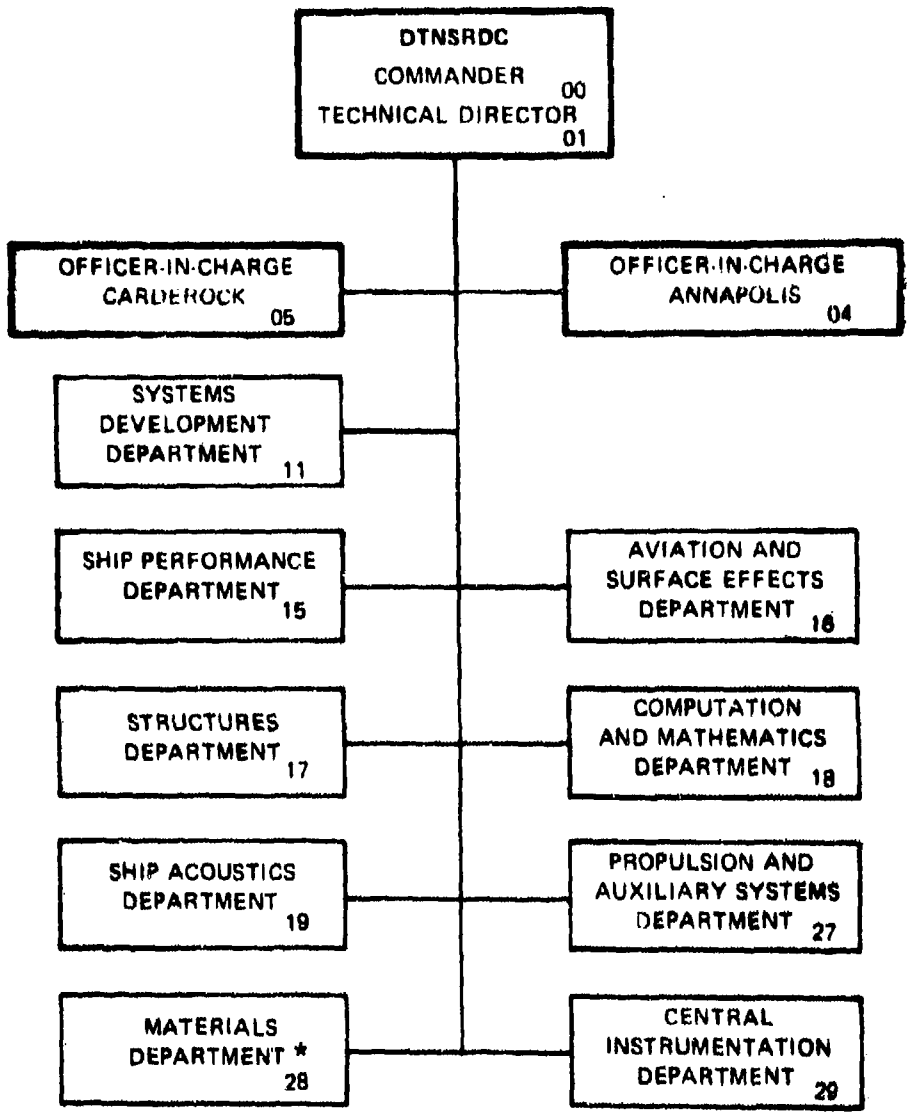

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The past decades have seen the emergence of enzyme technology for practical applications. Improvement of immobilized enzyme techniques has permitted enzyme-catalyzed industrial reactions, previously carried out in batch processes or prohibited by cost, to be scaled

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20. up for continuous operation and to show favorable economics. The food and pharmaceutical industries have been the pioneers of this technology. Other industries are presently investigating the potential benefits that may be derived from enzyme applications. The pollution abatement field, which has had to adapt new and existing technology to solve problems, has been especially receptive to new approaches such as enzyme catalysis.

A review of current enzyme technology and applications was undertaken. Five areas were identified where enzymes may provide solutions to shipboard waste management problems. They are: (1) waste treatment systems, (2) odor control, (3) cleaning operations, (4) oil pollution control, and (5) analytical/monitoring systems. These areas are discussed and recommendations for future research are presented.

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TABLE OF CONTENTS

ABSTRACT

The past decades have seen the emergence of enzyme technology for practical applications. Improvement of immobilized enzyme techniques has permitted enzyme-catalyzed industrial reactions, previously carried out in batch processes or prohibited by cost, to be scaled up for continuous operation and to show favorable economics. The food and pharmaceutical industries have been the pioneers of this technology. Other industries are presently investigating the potential benefits that may be derived from enzyme applications. The pollution abatement field, which has had to adapt new and existing technology to solve problems, has been especially receptive to new approaches such as enzyme catalysis.

A review of current enzyme technology and applications was undertaken. Five areas were identified where enzymes may provide solutions to shipboard waste management problems. They are: (1) waste treatment systems, (2) odor control, (3) cleaning operations, (4) oil pollution control, and (5) analytical/monitoring systems. These areas are discussed and recommendations for future research are presented.

INTRODUCTION

The Navy has been required, through the enactment of Executive Orders 11752 and 11514, to provide leadership in a nationwide effort to protect and enhance the quality of the environment. At present, the Navy is concerned with at least ten Federal Laws which are applicable to environmental protection.[1] This legislation together with the regulations of the Environmental Protection Agency (EPA), Coast Guard, and proposed International Maritime Consultative Organization makes it necessary for the Navy to adapt new and existing technology as well as to develop specific processes and techniques, where applicable, to comply with all environmental laws and regulations.

As a relatively new technology, enzymes have been referred to as a "solution in search of problems".[2] Enzymes may be applicable to solving shipboard waste management problems.

To assess the potential of enzyme technology for shipboard waste management, numerous sources of information were consulted.

[1] Superscripts refer to similarly numbered entries in the Technical References at the end of the text.

These include computer searches of Chemical Abstracts (CHEM. CONDENSATES), Chemical Patents (CAIMS-CHEM), Biological Abstracts (BIOSIS PREVIEWS), National Technical Information Service (NTIS), National Library of Medicine (MEDLARS), Bibliography of the National Agricultural Library (CAIN), Engineering Index, Institution of Mechanical Engineers Abstracts, Smithsonian Science Information Exchange, Water Resources Scientific Information Center (WATER RESOURCES ABSTRACTS), Oceanic Abstracts, Science Citation Index (SCI-SEARCH), and Comprehensive Dissertation Index. Only the most pertinent publications have been included in the reference section. In addition, discussions were held with industrial, academic, and government professionals possessing expertise in enzyme applications.

This report reviews current enzyme technology and discusses several areas in which enzymes may be beneficial for shipboard waste management.

BACKGROUND

It was almost a century ago (1878) that the German physiologist Wilhelm Kuhne introduced the term enzyme (meaning "in yeast") into the biological glossaries.[3] Since that time, enzymes have emerged from a laboratory curiosity to a valuable product for the food and pharmaceutical industries.

For centuries man has been enjoying the benefits of enzymes and their activity (bread, cheese, wine) without any knowledge or understanding of their functions. It has only been within the past few decades that research studies have been directed towards understanding the mechanisms of enzyme reactions. The earliest reported work on enzymes is credited to Payen and Pessoz who showed that the liquefaction of starch could be achieved with a material they called diastase.[4] Several years later, Berzelius noted the catalytic effect that occurs in biological reactions and predicted this phenomenon in all living cells.[5] It was not until 1926 that Sumner in his work in isolating and purifying urease from the jack bean showed this enzyme to be a protein molecule. Numerous investigators have since determined that all enzymes known to date are indeed proteins, consisting of chains of amino acids, and this finding is now universally accepted. Over the past 70 years, five Nobel prizes have been awarded to investigators for their work in enzyme related developments. However, despite all of these contributions and achievements, the mechanisms of enzyme action are not yet completely understood.[4]

ENZYME CHARACTERISTICS

Enzymes are biological catalysts that accelerate virtually all of the known chemical reactions occurring in living cells. These reactions, due to the relatively high energies of activation that are involved, would not be able to sustain the metabolic processes of the cells were it not for the presence of enzymes. The enzymes, presumably, lower the activation energy necessary for the reaction to proceed.[5]

All naturally occurring enzymes are either found within or secreted from living cells. Those that are produced within the cell (the majority of enzymes) are referred to as intracellular enzymes, while those that are formed within the cell but excreted are known as extracellular enzymes. Both are essential in maintaining the metabolic processes of living cells.

As proteins, enzymes exist as compounds ranging in molecular weight from about 10,000 to several million. There are several enzymes known to consist solely of proteins, such as the digestive enzymes pepsin and trypsin. However, most enzymes are more complex, consisting of an apoenzyme (protein portion) and a cofactor (nonprotein portion). Together, these two components are known as the holoenzyme. The cofactors may be either a low molecular weight nonprotein organic molecule or a metal cation. If the nonprotein is loosely attached to the apoenzyme, it is called a coenzyme and is available for attachment to other apoenzymes in the cell to accomplish the same reaction. If the nonprotein is firmly attached to the apoenzyme it is referred to as a prosthetic group. Metal ions such as magnesium, iron, zinc, etc., serve as enzyme activators bringing the substrate (substance to be transformed) together with the enzyme to permit the reaction to proceed.

The substrate bonds to an area of the protein portion of the enzyme called the active site (see figure 1). It is within this active site of the enzyme that an enzyme-substrate complex is formed, which eventually breaks down into a product and an unaltered enzyme (depending upon the equilibrium, there may be a regeneration of substrate and enzyme). Less than 10% of the overall structure of the enzyme is active. The active sites are generally found in the middle of the conformational structure with polar groups observed on the outside and the nonpolar groups on the inside. The remaining 90% of the enzyme structure (nonactive portion) provides a protective function, maintains the protein structure, and binds to other cell components (enzymes, membranes, reticula, etc.).

Enzymes have been divided into six main classes according to a system developed by the International Union of Biochemistry.[a] This classification, including the enzyme's catalytic functions, is shown in table 1.

TABLE 1
CLASSES OF ENZYMES AND THEIR FUNCTIONS

Enzyme Class	Functions
Oxidoreductases	Oxidation-reduction reactions
Transferases	Group transfer reactions
Hydrolases	Cleave molecules with the uptake of water
Lyases	Removal of groups or addition to double bonds
Isomerases	Isomerization reactions
Ligases	Joining of molecules

Some of the earliest enzymes investigated have retained their original names, e.g. pepsin, trypsin, chymotrypsin, papain. However, with the current nomenclature system, the suffix -ase is added to the name of the substrate the enzyme acts upon or of the reaction the enzyme will be accelerating. Thus, the enzyme that causes the degradation of urea would be called urease while the enzyme that promotes the breakdown of cellulose would be called cellulase. The enzymes that take part in oxidation reactions would be named oxidases and those that hasten reduction reactions are known as reductases.

As proteinaceous substances, enzymes are susceptible to the same physical and chemical environments that affect proteins. Consequently, high temperatures, generally in excess of the 40°-50° C range, will cause the destruction or denaturation of most enzymes, while low temperatures, less than 5° C, will inhibit or arrest their activity without generally causing conformational change. Some heat resisting (thermophilic) microorganisms have enzymes capable of withstanding temperatures in excess of 70° C while some Arctic bacteria have enzymes that exhibit optimum activity at 0° C.[b] Figure 2 illustrates the effect of temperature on the enzymatic reaction rate. For approximately every 10° C rise in temperature, to about 40° C, the rate of most enzymatic reactions will double.

The optimum activity of enzymes is also affected by pH, which varies for different enzymes as well as for different substrates. For example, the digestive enzyme pepsin has an optimum activity at a pH of 1.5-1.6 while the enzyme lipase functions optimally in the pancreas at a pH of 8.0. As the pH varies to either side of the optimum pH, i.e., at high hydrogen ion or hydroxyl ion concentrations, the enzyme will be denatured. Figure 3 shows the

effect of pH and the enzymatic reaction rate. Metals such as magnesium, iron, zinc, and copper and high concentrations of salts such as sodium chloride and ammonium sulfate, adversely affect enzyme reactions by precipitating the protein portion of the enzyme.

Both enzyme concentration and substrate concentration affect enzymatic reactions. There appears to be an optimum concentration for each. This is shown graphically in figures 4 and 5.

A characteristic of enzymes which is unique in catalysis is the specificity enzymes exhibit toward particular substrates or particular chemical reactions. Certain enzymes will catalyze only one type of reaction while others will exhibit specificity for a particular chemical bond or functional group of a molecule. Enzymes possess the inherent capability of accelerating reactions by more than 10^{11} times over the corresponding nonenzymatic reaction. At this increased rate, a given enzyme molecule may convert from 10^{3}-10^{6} substrate molecules per minute (the turnover number or the number of substrate molecules metabolized per enzyme molecule per minute.) The enzyme catalase, found in red blood cells, has one of the highest reported turnover numbers, 1.8×10^{7}. As in the case of inorganic catalysts, enzymes will emerge from a given reaction without being consumed, although both are affected by attrition.

ENZYME KINETICS

No discussion on enzymes would be complete without a brief description of enzyme kinetics. The following is a simplified derivation of the Michaelis-Menton equation. Use is made of two fundamental assumptions: (1) under steady-state conditions the enzyme exists as either the pure enzyme, E, or in the complex, ES (where S is the substrate), and (2) the complex ES is in equilibrium with the enzyme and substrate.

The chemical equation for an enzymatic reaction is generally expressed as:

$$E + S \rightleftharpoons ES \rightleftharpoons P + E \qquad (1)$$

where E, S, and P are the enzyme, substrate, and product concentrations, respectively, and ES is the intermediate enzyme substrate complex. The existence of this complex has been demonstrated by spectral data which show bands different from those produced by either product or substrate. If the reaction rate constants are included, equation (1) may be written as:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} P + E \qquad (2)$$

where k_1 and k_2 are the forward reaction rate constants and k_{-1} and k_{-2} are the reverse reaction rate constants. Generally, k_{-2} is negligible and is omitted.

Using the law of mass action to describe the change in the ES complex with time produces equation (3):

$$\frac{d[ES]}{dt} = k_1 [E][S] - k_{-1}[ES] - k_2[ES] \quad . \qquad (3)$$

The total concentration of enzyme, free or complexed, may be defined as E_t, such that,

$$E_t = E + ES \quad . \qquad (4)$$

Since at steady state,

$$\frac{d[ES]}{dt} = 0 \qquad (5)$$

rearranging equation (3) results in:

$$[ES] = \frac{k_1[E_t][S]}{k_1[S] + k_2 + k_{-1}} = \frac{[E_t][S]}{[S] + \frac{k_2 + k_{-1}}{k_1}} \quad . \qquad (6)$$

Recognizing that the rate constant term in equation (6) may be defined by an overall constant, K_m, such that

$$K_m = \frac{k_2 + k_{-1}}{k_1} \quad , \qquad (7)$$

equation (6) may then be rewritten as

$$[ES] = \frac{[E_t][S]}{[S] + K_m} \qquad (8)$$

where K_m is the Michaelis constant.

It follows from equation (2) that the rate of product formation is dependent upon the concentration of the enzyme-substrate complex ES; that is

$$\frac{d[P]}{dt} = k_2[ES] \quad . \tag{9}$$

If

$$v = \frac{d[P]}{dt} = k_2[ES] \tag{10}$$

then, substituting this expression into equation (8),

$$v = \frac{k_2[E_t][S]}{[S] + K_m} \quad . \tag{11}$$

By defining

$$V_{max} = k_2[E_t] \tag{12}$$

hence, the Michaelis-Menton equation

$$v = \frac{V_{max}[S]}{[S] + K_m} \tag{13}$$

where V_{max} is the maximum velocity under a given set of conditions. Since equation (13) can be rewritten as a linear equation (y=mx+b), then,

$$V_{max}[S] = vK_m + v[S]. \tag{14}$$

The Michaelis-Menton equation becomes a valuable tool in enzyme assay for predicting the conditions for which the velocity will be linear with either substrate or enzyme concentration.

ENZYME REACTORS

Reactor design procedures for enzymatic catalysis are similar to those required for conventional catalysis. Reactions are carried out as either batch or continuous operations. Batch reactors (stirred tanks) offer flexibility, simplicity, and low capital investment but incur high operating labor costs because of the separations required on shutdown, i.e., separation of enzyme, product, and unreacted substrate. Continuous reactors require high capital investment and expenditures but avoid the high

operating labor costs by functioning at steady-state conditions. Consequently, improved conversion rates, yields, and enzyme stability are attained. Continuous reactors can be readily automated and scaled up.

Two types of continuous reactors are generally considered for enzyme reactions. They are the continuous stirred tank (CSTR) and the tubular (plug flow) reactors. Efficiencies of these reactors have been summarized by Dunn.[7] The CSTR is advantageous at high product and low substrate concentrations while the tubular reactor shows the best efficiency with high substrate and low product concentrations. Furthermore, Dunn also has shown that the CSTR is the best choice when enzyme reactor systems are inhibited by the substrate while the tubular reactor is the best choice when the reactor systems are inhibited by the product or when the reaction follows Michaelis-Menton kinetics.[7]

Another design consideration for enzymatic reactors is that of using soluble or insoluble enzymes. Soluble enzymes may be used in either batch or continuous operations. In the batchwise process, the soluble enzyme is removed and discarded during product purification. However, recent technology has enabled soluble enzymes to be reused in continuous reactors with ultrafiltration membranes. Ultrafiltration is a pressure-driven membrane process that offers the ability to separate, fractionate, and concentrate macromolecules and suspended solids from solutions, or suspensions (such as wastewater), while allowing low molecular weight and ionic species to pass through the membrane. Manufacturers of ultrafiltration (UF) membranes currently offer membranes that can be tailored to reject the enzyme and substrate while allowing the smaller product molecules to permeate. Thus, the separation step inherent in the batch operation is eliminated in the continuous operation, and the membrane retains the soluble enzyme and unreacted substrate for further reaction.

An alternative to the soluble enzyme-ultrafiltration process is the application of insoluble enzymes, generally referred to as immobilized enzymes. This concept will be discussed in more detail in the next section. A recent review of enzyme engineering listed the advantages of immobilized enzymes over soluble enzymes.[8] A summary of this review is shown in table 2.

TABLE 2
ADVANTAGES OF IMMOBILIZED ENZYMES
OVER SOLUBLE ENZYMES

• The enzymes can be reused. • Continuous processes become practical. • Improved stability of enzymes. • Greater purity of products. • More favorable or broader environmental stability (e.g., pH and temperature.) • Can possibly be tailor-made for specific end-use. • Enzyme activity is sometimes enhanced. • Minimizes effluent problems and materials handling.
Source: Vieth, et.al., p. 678.[5]

Either batch or continuous reactors may be used with immobilized enzymes. Batch reactors require a separation technique to recover for reuse the enzymes which are immobilized on solid supports. However, for the continuous process, numerous alternative reactor designs are available. A CSTR is the simplest continuous design but presents the same problems encountered with the batch reactor. Enzymes can be immobilized to UF membranes rather than used in their soluble form. Extensive research is currently underway in various universities and industries to perfect this technique. Packed beds of immobilized enzymes are practical and efficient enzymatic reactors but are susceptible to operational problems (plugging, attrition). Another concept involves passing the wastewater through a fluidized bed which contains enzymes immobilized on solid carriers.

In summary, to choose an enzyme reactor for a specific application, the design engineer should carefully investigate the kinetics of the reaction, the operational requirements, the capital costs of equipment, and finally determine the advantages and disadvantages of recovering and/or reusing the enzyme.

ENZYME IMMOBILIZATION

Immobilization is defined as "the physical confinement or localization of enzyme molecules during a continuous catalytic process."[9] This would also include enzymes that are chemically or physically attached to various cell components such as the nuclear membrane, endoplasmic reticulum, mitochondria, etc. For purposes of this discussion, however, only enzymes that are nonbiologically immobilized are considered.

Enzyme immobilization methods may be grouped into two major classes - chemical and physical. Chemical methods are those that involve the formation of covalent bonds between enzyme residues (functional groups) and a support material. These methods generally reduce enzyme activity due to reactions affecting essential amino acid groups. In most cases the enzyme cannot be recovered in its original state. Physical methods involve the localization of an enzyme on a support material that is not dependent on covalent bond formation.[9] Table 3 summarizes the chemical and physical methods employed for enzyme immobilization.

TABLE 3
IMMOBILIZATION METHODS

Chemical Methods (Covalent Bond Formation - Dependent)
• Attachment of enzyme to water-insoluble, functionalized polymer.
• Incorporation of enzyme into growing polymer chain.
• Intermolecular crosslinking of enzyme with multifunctional, low molecular weight reagent.
Physical Methods (Noncovalent Bond Formation - Dependent)
• Adsorption of enzyme on water-insoluble matrix.
• Entrapment of enzyme within water-insoluble get matrix (lattice-entrapment).
• Containment of enzyme within special semipermeable membrane-dependent devices.
Source: Zaborsky, page 3.[9]

The most common chemical method for immobilizing enzymes is the covalent attachment of water-soluble enzyme molecules to water-insoluble, functionalized organic or inorganic polymer supports. Table 4 is a list of some of the more common supports used. As Zaborsky[9] states, enzyme immobilization "should involve only functional groups of the enzyme that are not essential for catalysis and those if reacted, would not detrimentally alter any chemical or physical property of the enzyme."

TABLE 4
COMMONLY EMPLOYED WATER-INSOLUBLE SUPPORTS
FOR THE COVALENT ATTACHMENT OF ENZYMES

Synthetic Supports
Acrylamide-based polymers Maleic anhydride-based polymers Methacrylic acid-based polymers Polypeptides Styrene-based polymers
Natural Supports
Agarose (Sepharose) Cellulose Dextran (Sephadex) Glass Starch
Source: Zaborsky, page 9.[9]

The adsorption of enzymes on surfaces is the most common physical method of immobilization. This method consists of contacting an aqueous enzyme solution with a surface-active organic/inorganic adsorbent or support. Some commonly used supports include alumina, ion exchange resins, carbon, cellulose, and clays.[9] Advantages of adsorption are relative simplicity, large choice of supports and the possibility of purifying the enzyme while localizing it. Disadvantages include the precise pH, temperature, and ionic strength control required if strong binding is to be achieved.[9]

Another physical method is entrapment within crosslinked polymers. In this approach, the enzyme molecules are physically contained within a crosslinked network of a polymer. The enzyme cannot leave the gel matrix, but substrate and product molecules can move across and within the network to ensure continuous reaction. The most common entrapment matrix is polyacrylamide; however, silica gel, starch, and silastic resin are also used.[9]

Microencapsulation is a physical method of immobilization in which enzyme molecules are trapped within microcapsules that have either a permanent or nonpermanent semipermeable membrane. Nonpermanent membranes are also known as "liquid-surfactant membranes." The principle of operation is based on the selective permeability of the membrane. The enzyme molecules are larger than the pore diameter of the spherical membrane and cannot diffuse out. The substrate molecule, whose size does not exceed the pore diameter,

can readily diffuse through the membrane, and be transformed to product by the entrapped enzyme molecules. The reaction product then diffuses through the membrane to the exterior. Only substrates and products of relatively low molecular weights can be considered with this approach. The most common membranes used in microencapsulation are nylon, silicone polymers, and collodion.[3]

CURRENT APPLICATIONS

The widest application of enzymes has resided with the food and drug industries, as seen in table 5.

TABLE 5
COMMERCIAL APPLICATIONS OF ENZYMES

Enzyme Type	Applications
Soluble Enzymes	
Amylase	Starch degradation; conversion of starch to glucose; liquefaction of grain; removal of starch sites.
Protease	Precipitation of casein for cheese making; tenderizing meat; digestive aid with food; loosening hair for leather manufacturing; chillproofing beer in brewing.
Cellulase	Clarification of fruit juices.
Oxidase	Removal of glucose or oxygen.
Immobilized Enzymes	
Penicillin amidase	Synthetic penicillin
Glucose isomerase	Conversion of starch to glucose.
Acylase	Production of the amino acid L-methionine.

Conversion of sucrose to the sweeter invert sugar by the enzyme invertase is a well established process, as is the conversion of starch to glucose by the enzyme glucoamylase. Both of these processes have employed soluble enzymes. The present trend in industrial operations, where enzymes are used to accelerate product formation rates, is to incorporate immobilized enzyme technology. Glucoseisomerase has recently been used in the immobilized form for converting corn starch to high fructose corn syrups, a product equivalent in sweetness to the conventional cane and beet sugars.[2] Semisynthetic penicillins and various amino acids

are presently produced by use of immobilized enzymes in the United States, England, and Japan.[2,10] An immobilized enzyme process that can produce over 10 tons (9.1 megagrams) per month of the amino acid L-methionine was recently described by Bungay.[11]

One of the inherent properties of enzymes is their unique ability to catalyze specific reactions. This characteristic has been valuable in the detection and quantification of various compounds in aqueous solutions, such as using enzymes to measure trace contaminants in water.[12] Both soluble and immobilized enzymes have application in clinical diagnosis.[13] The addition of enzymes to samples of body fluids can be used to determine the concentration of specific components of that fluid. Recently, analytical instrumentation utilizing immobilized enzymes has been developed for the detection of glucose while another has been used to determine urea nitrogen.[2] Biosensors, employing an enzyme electrode concept, have been reported for detecting the substrates adenosine monophosphate (AMP),[14] cholesterol, and glucose.[15] Miles Laboratories is currently developing a nylon-immobilized enzyme tube for use with automated analyzers.

Attempts have also been made to utilize enzymes in waste treatment technology. Difficulties have been encountered, however. Slote,[16] in a study (funded by the EPA) to enhance the conventional biological treatment process, reviewed previous investigations and proposed explanations for their deficiencies:" (1) the enzymes were crude preparations and were not isolated from the native microorganisms involved in the activated sludge process, and (2) no attempt was made to protect the enzymes from rapid destruction by the viable microorganisms in the activated sludge to which they were added." Although the study attempted to overcome these problems by isolating, characterizing, purifying, and immobilizing enzymes found in activated sludge flocs, difficulties were experienced with the immobilized enzyme technique employed (encapsulation). Results of this investigation were thus inconclusive. In contrast with this, currently available brochures describe how the addition of powdered bacteria/enzyme preparations can enhance the biological treatment rate by as much as 25%. Hankin and Sands recently suggested the possible use of stabilized enzymes for enhancing the biological treatment process.[17]

Several studies concerned with the ability of enzymes to hydrolyze waste sludges have been cited recently.[18] The Army's Natick Laboratory has developed an enzyme treatment process which utilizes a mixture of soluble and immobilized cellulase enzyme to degrade cellulase to glucose. The sugar produced could be used as a food source or broken down further into alcohol which could be

recovered as a fuel. Other Army research has shown enzyme/bacterial cultures to be capable of at least altering explosive waste compounds.[19] Enzymes have also been found effective for disinfecting viruses in wastewater although the rate of kill is low.[20]

Other applications that have been reported for enzyme utilization in waste treatment processes include: the conversion of dairy waste effluents (whey) into food supplements,[10] the degradation of citrus wastes into low caloric, nontoxic sweeteners,[10] the degradation of phenol by phenoloxidase,[10] which also could be used as a monitoring technique,[11] and the removal of viruses by air disinfection with deoxyribonucleases.[11]

Certain enzyme products, marketed primarily to enhance waste degradation, also have been found to be effective in controlling odors emanating from sewage treatment systems. Enzymatic odor control is new to the scientific community and virtually all information on this subject comes from enzyme manufacturers. Recent experience at this Center has indicated that some of these proprietary enzyme/bacterial products can indeed enhance odor control.

Application of enzymes for the control of oil pollution has seen only limited use. Most of the reported work is concerned with the microbial degradation of oil by bacterial and yeast cultures. Numerous practical applications for controlled microbiological processes have been demonstrated; however, they are still in an early stage of development and the enzymatic systems and responsible organisms have not been defined.[21]

The Navy, the Department of Transportation (Coast Guard), and the EPA have sponsored a number of studies concerned with the feasibility of using microorganisms for the degradation of petroleum products. These studies have focused on the identification and characterization of microorganisms that have the ability to degrade hydrocarbons. More than 200 species of bacteria, yeasts, and filamentous fungi have been shown to metabolize one or more kinds of hydrocarbons ranging from methane (CH_4) to compounds containing more than 40 carbon atoms.[22] Each species generally metabolizes only a narrow spectrum of homologous hydrocarbons; however, in many species, the enzymes that catalyze the oxidation of hydrocarbons are adaptable or can be induced.[23] A number of pure cultures have been developed to attack petrochemicals by cultivating them in appropriate heterotrophic media enriched with one or more hydrocarbons.[22]

The emulsification of oil in an aqueous system renders it more susceptible to enzymatic attack. Some microbial species produce cofactors (surfactants) which tend to emulsify oil in water.[23,24] These cofactors have been studied along with some commercially available, proprietary bacterial products which have achieved limited, and in some cases, disputed success.[24] For example, in a study on the effect of some commercial bacterial inocula on the biodegradation of oil in seawater, the investigators found the two products tested to be totally ineffective in stimulating biodegradation, both in terms of degradation and the rate or extent of mineralization.[25] A study concerned with the biodegradation of crude oil in ballast water, conducted on a tanker during its ballast voyage, showed that after 4 days of aeration of oily ballast water there was no sign of oil.[26] In another study using bacterial seeding to enhance biodegradation of oil slicks, it was observed that the seeding did enhance the degradation process.[27,28] These experiments revealed that the effectiveness of microbial seeding varied more with type and quantity of crude oil used than with such factors as inoculum density or nutrient concentration.[27] In this same study, it was noted that mixed bacterial cultures more effectively degraded a variety of crude oils and pure hydrocarbons than did single isolates. This observation is consistent with the conclusions drawn by other investigators.[28,29,30]

The potential for enzyme applications is "unlimited" and can best be summarized by the following statement: "If we were able to use a greater fraction of the remarkable potentials of enzymes we might well be able to revolutionize the chemical industry and many aspects of our health and daily lives. Recent advances in fundamental and applied enzymology indicate that we have already started in that direction. At a time when renewable resources are increasing rapidly in importance, it is indeed a welcome sign."[31]

POTENTIAL NAVAL APPLICATIONS

Listed below are five areas where Navy shipboard waste management may benefit from enzyme technology.

- Wastewater treatment.
- Odor control.
- Oil pollution control.
- Cleaning agents.
- Analytical/monitoring.

These potential applications are not limited to Navy problems, but are also relevant to solving problems encountered on any vessel, or for solving land-based problems as well. Many of the above areas are interrelated in that the use of enzymes for one application may result in benefits to another; e.g., wastewater treatment and odor control. The following discussion attempts to point out where enzymes may be beneficial for shipboard waste management and what research and development efforts are required to establish practicable systems.

WASTEWATER TREATMENT

Present Navy policy includes the installation of sanitary waste collection, holding, and transfer (CHT) systems on most ships of the Fleet by 1980. However, several ships (LHA's) will be equipped with a biological treatment system. Enzyme/bacterial addition, as previously discussed, has been shown to increase the biological treatment rate by 25%. Greater treatment rates could be obtained with increased enzyme concentration but at higher cost. Such rate increases would result in either a smaller treatment system design or the ability to treat more waste with an existing system in a given period of time. The addition of these enzyme preparations should be evaluated further to establish optimum rates versus cost.

One method available for potentially lowering the enzyme cost while at the same time increasing treatment efficiency is enzyme immobilization. The utilization of a packed bed immobilized enzyme system (with glass beads or media similar to that used in the trickling filter design, where more intimate contact is provided for organic substrate consumption) should be considered as a more practical alternative to the encapsulated gel approach previously mentioned. The enzyme/bacterial preparation or a formulation from an activated sludge floc could be immobilized for continuous treatment. Either of these concepts could ultimately result in greater treatment efficiency, favorable economics, and reduction in manpower requirements to make the modified biological treatment concept acceptable for shipboard waste management.

Roughly 50% of the dry weight of the solids of mixed domestic sewage is composed of cellulose. In the biological treatment process the microorganisms tend to consume this substance slowly and it is still being degraded long after the other organic substrates have been metabolized. If the current work in cellulose degradation, employing the enzyme cellulase, could be utilized with the biological treatment process, the cellulosic material could then be solubilized and eventually degraded more rapidly. However, it should be noted that a digestion step (usually hot

alkali) is required before the cellulase can perform its enzymatic function. Present discharge requirements for sewage have limits only for suspended solids and fecal coliform. In anticipation of stricter regulations in the future, soluble organics would be far less troublesome to treat and dispose of than the suspended matter. Consequently, research and development efforts in enzymatic conversion of the cellulosic fraction of sewage wastes into a soluble form should be explored.

One of the processes the Navy is presently evaluating for treating raw sewage is UF using noncellulosic membranes. A problem encountered in testing this pressure-driven membrane process is that of membrane fouling. This phenomenon, associated with the buildup of gelatinous materials at the surface of the membrane results in a subsequent decrease in its permeation rate (flux). If this fouling layer could be modified or eliminated, the efficiency and reliability/maintainability of the UF system would be significantly improved.

A concept that is being investigated by the Navy to enhance UF performance involves using commercially available enzymes immobilized within or near the membrane surface to degrade the gelatinous buildup as it is formed. Initial studies have shown that this gelatinous layer consists mostly of proteins and lipids. Attempts to employ proteases and lipases to degrade this layer in static tests have been successful. A determination of the optimum enzyme system which would be immobilized on noncellulosic membranes is required. Immobilized enzyme technology appears to have advanced significantly over the past decade to the point where the selected enzymes can be successfully attached to the membrane to demonstrate the proposed concept.

Organotin compounds are currently used in some Navy antifouling paint formulations. Periodically, paint coatings must be stripped and restored in order to maintain the antifouling properties. During this removal operation, significant quantities of toxic organotin-laden wastewater is generated. Detoxification of this wastewater must be achieved if a potentially adverse impact on the environment is to be avoided.

One potential method for detoxifying these organotin compounds is through enzymatic reactions. Investigations with an organotin fungicide (tributyltin acetate) have shown this substance to be biodegradable in soil,[32] although the rate is slow. A more recent study with several tributyltin compounds using a mixed function oxygenase resulted in the formation of several hydroxylbutyldibutyltin derivatives.[33] Tributyltin oxide is toxic to fish at concentrations as low as six parts per billion. Consequently, the application of enzymes for detoxifying organotin compounds found in

antifouling paints should be investigated. A good starting point would be to examine soils and sands that have been in contact with the organotin compounds for biological activity and breakdown products containing tin.

ODOR CONTROL

Another area for potential enzyme application is in the control of odors emanating from sewage holding tanks, sewage transfer barges, sewage hoses, lines and connections, waste treatment systems, small craft sanitation devices, bilges, and oil/water separator systems.

Most objectionable odors from wastewater and waste treatment systems are classified as either inorganic gases or organic vapors.[34] The organic vapors are mainly derived from the anaerobic decomposition of nitrogen and sulfur-containing compounds but may also result from the presence of industrial waste chemicals.

The addition of enzyme/bacterial products for the enhancement of waste degradation has in some cases resulted in reduction of sewage odors. The exact mechanism for enzymatic odor control is not well known or understood. It may be that the enzymes convert certain odorous compounds into nonodorous forms, or that they convert a specific substrate required for odor production into a product that will not result in odor formation. Certain enzyme reactions may produce substances that combine with the odorous compound in such a way that a "neutralization" occurs and the odor is not detected.[34,35]

It is known that some strains of bacteria produce enzymes that use dissolved gases as substrates; e.g., the enzyme oxidase can oxidize hydrogen sulfide (H_2S) to sulfate and elemental sulfur. Air filtration systems activated with appropriate enzymes could be used in vents or stacks that discharge odorous gases from waste holding tanks and treatment systems. A similar approach may be used to treat odors in oil/water separator systems. In the case of H_2S produced in bilges, it may be more desirable to halt or limit gas evolution rather than treat the gas after it has been produced. Once H_2S has been formed in the bilge it is generally dissolved, depending on such factors as pH, temperature, and other constituents present. Bilges are generally coated with coal-tar epoxy paint and are, therefore, not susceptible to the corrosive effects of the dissolved H_2S. When the bilges are pumped, however, the dissolved H_2S (hydrosulfuric acid) may then attack uncoated pump interiors and pipes and cause their eventual deterioration. Furthermore, when H_2S gas is exposed to a moist

aerated environment (such as the space above the bilge water level), it may be biologically converted to sulfuric acid (H_2SO_4). This acid is substantially more corrosive than the dissolved H_2S.

One approach to overcome this problem would be to add enzymes or bacterial cultures producing the enzymes directly in the bilges to oxidize the H_2S to sulfate as the gas is produced. Laboratory and shipboard evaluations need to be conducted concerning the ability of enzyme/bacterial products to reduce or eliminate odors in shipboard waste systems. Screening studies should be undertaken to determine the ability of enzymes to function under varying environmental conditions (temperature, pH), its method of application, and its cost.

CLEANING AGENTS

A number of proprietary enzyme/bacterial preparations which are available commercially are being used in cleaning operations. For example, cleaning grease traps and drain lines, equipment in rendering plants, and septic and sewage systems are typical applications. Preliminary tests conducted recently by the Center aboard USS DIXON (AS-37) have revealed that the addition of a dried bacterial culture resulted in the removal of large deposits of grease and scale from the walls and pipes in a waste holding tank after 10 days of treatment. It appears that addition of such enzymes or bacterial cultures which produce the desired enzymes can save hours or days of labor in meeting maintenance requirements while indirectly minimizing the risk of health hazards to personnel involved in tank-cleaning operations. It is anticipated that periodic replenishment of the enzyme/bacterial culture would be required due to losses to be expected during pump out operations. Consequently, tradeoff studies need to be undertaken to determine whether the frequency of product addition to obtain the desired cleaning level is feasible. Studies are required to evaluate commercially available enzyme/bacterial products for their ability to clean holding tanks receiving laundry, shower, sewage, and food wastes. Shipboard evaluation of these products should be conducted under realistic conditions and should be preceded by laboratory screening experiments.

OIL POLLUTION CONTROL

Shipboard application of enzyme/bacterial cultures for oily wastes appears to be limited primarily by (1) the time element (the complete degradation of oil by enzymatic reaction requires days to complete) and (2) the generation rate of bilge water coupled with the limited storage capacity aboard ship. In recent years, the Navy has devoted a considerable effort in the development

and evaluation of mechanical oil/water separation systems. These devices have demonstrated an ability to separate oil from water.

Until recently, it was believed that no one species of bacteria could degrade all forms of petroleum products and that specific bacterial cultures would have to be tailored to the specific type of oil to be degraded. This approach made enzyme/bacterial utilization appear exotic and in many cases impractical. However, a strain of pseudomonas bacteria has recently been genetically altered so that it is capable of synthesizing enzymes which accelerate the oxidation of aliphatics, aromatics, polynuclear aromatics, and terpenes,[36,37] nearly all the hydrocarbons found in petroleum. If these enzymes can be extracted and immobilized, research efforts should be undertaken to determine the feasibility of utilizing enzymes in shipboard situations where time is not a factor, as in the case of ballast tanks filled with oily water during a ballast voyage. Such an application may be accomplished either batchwise with the direct addition of enzyme/bacterial preparations to the ballast tanks or through the development of enzyme reactor systems wherein the oily wastewater could be treated. Immobilized enzyme techniques may be useful in the latter application in a recirculating system. This approach would ultimately lead to savings in enzyme cost.

ANALYTICAL/MONITORING

One of the inherent properties of enzymes is their unique ability to catalyze specific reactions. This characteristic may be advantageous for shipboard application through the development of enzyme electrodes for the detection and quantification of pollutants.

Development of a method for the real-time determination of oil content of water is part of a current program of the Navy. Two problems recognized with monitors are the requirement of a skilled operator and the interference produced by solids, detergents or persistent oil films. Since enzymes possess a high degree of specificity and sensitivity, the problems exhibited by the present monitoring devices could be overcome with the development of enzyme-electrode monitors. Adaptation of current clinical diagnostic technology using enzymes for quantifying specific components of a fluid could accelerate the effort. A recent study has established the feasibility of such a technique and consequently, further development efforts are warranted.[38]

Another potential area for enzyme electrodes is the quantification of the detergent concentration in waste laundry water. Although no regulations or standards for discharging detergents

exist they are anticipated for the future. Furthermore, with recent attention being directed toward water reuse, it is possible that a treated laundry wastewater effluent could be recycled in the laundry process. In either case, it would be important to know the concentration of detergent (anionic or nonionic) in the discharge or recycle water. Since present analytical techniques for detergent determination are subject to interferences or are too tedious, development of an enzyme electrode for detergent detection and quantification would be extremely useful.

Another possible application for enzyme electrodes is in the detection of fecal coliform bacteria. The EPA and Coast Guard have established regulations for sewage discharged from marine vessels. One of these requirements is for fecal coliform bacteria. This is $\leq$1000 fecal coliform per 100 millilitres, but by January 1980 will be $\leq$200 fecal coliform per 100 millilitres. Existing methods for measuring fecal coliform require 24-96 hours. During this time span, large quantities of bacteria would be discharged before any changes could be made in the operation of a sewage treatment system.

A real-time fecal coliform detector based on an enzyme electrode concept would overcome this time delay problem. However, much research is necessary to determine specific substances that are present exclusively in fecal coliform but are not present in any of the other microorganisms in raw sewage. Furthermore, an enzyme would have to be identified which would convert this substance into compounds that can be readily detected and quantified for monitoring purposes. Development of such a monitor would not only directly benefit the Navy but would also have worldwide application in wastewater treatment plants.

SUMMARY AND RECOMMENDATIONS

An attempt has been made to review modern enzyme technology with respect to both fundamentals and applications. Five pertinent areas have been identified where enzymes may be applied to solve shipboard waste management problems. These are: (1) wastewater treatment systems, (2) odor control, (3) cleaning operations, (4) oil pollution control, and (5) analytical/monitoring systems. In order to develop these areas into practicable shipboard systems, specific research and development investigations are necessary. The following recommendations are summarized from the text:

- Develop a shipboard wastewater treatment system based on immobilized enzyme technology.

- Study the immobilized enzyme concept with UF membranes for preventing membrane fouling when separating and concentrating shipboard wastes.

- Identify and evaluate commercially available enzyme/bacterial products for odor control and cleaning operations in a shipboard environment.

- Determine the feasibility of adding enzyme/bacterial products for controlling oil discharges from ballast tanks.

- Investigate the application of enzymes for detoxifying organotin compounds produced during paint removal operations.

- Develop enzyme electrodes to monitor oil, detergent, and fecal coliform bacteria for (near) real-time measurement of these shipboard-generated pollutants.

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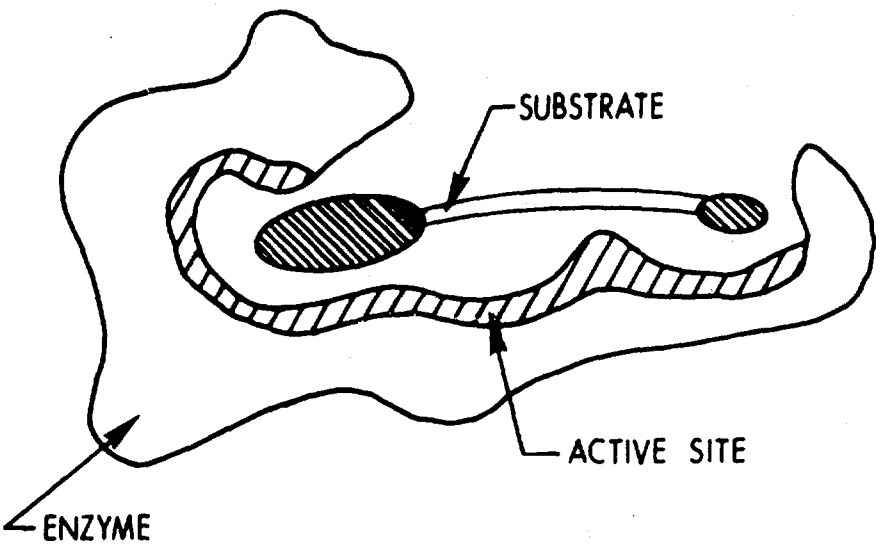

Figure 1
Active Site/Substrate Complex

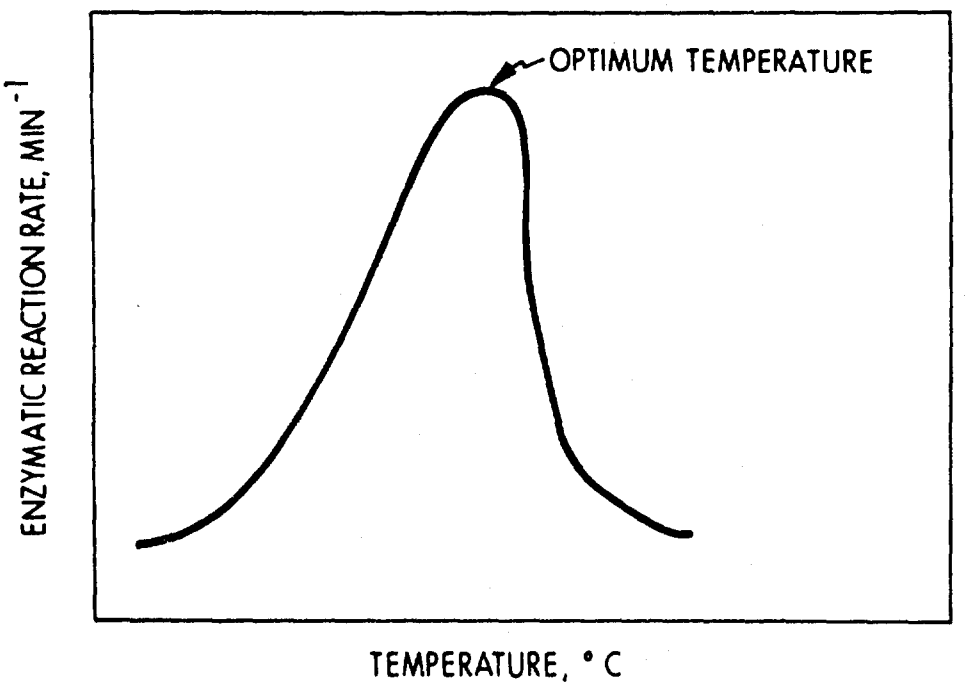

Figure 2
Effect of Temperature on Enzymatic Reaction Rate

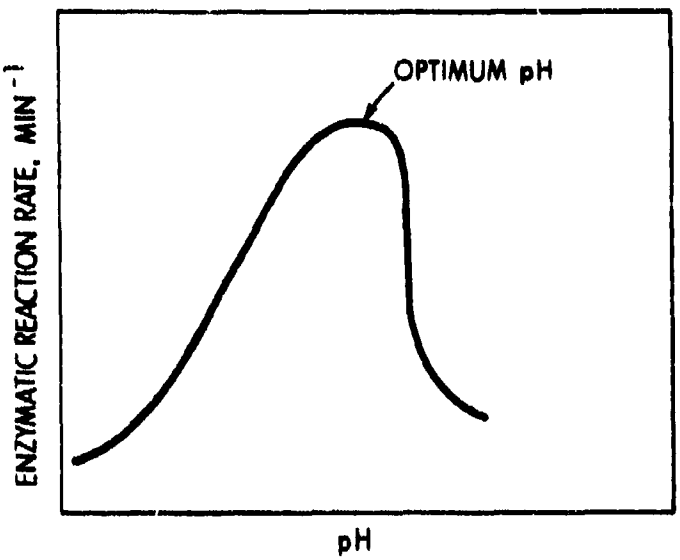

Figure 3
Effect of pH on
Enzymatic Reaction Rate

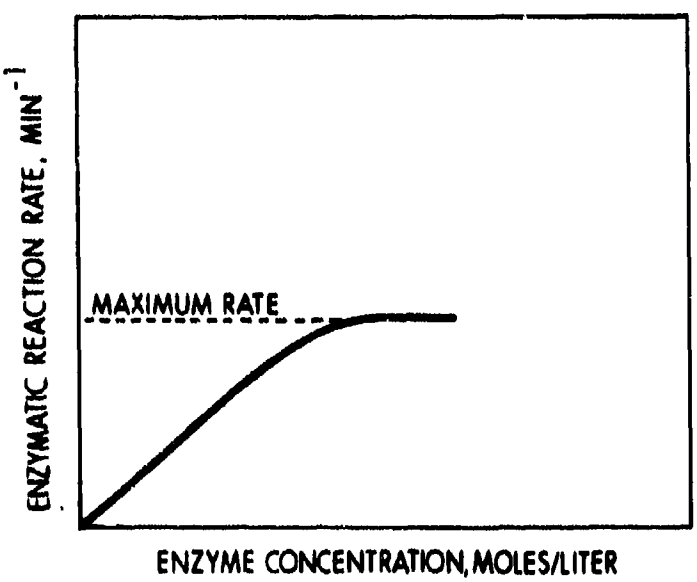

Figure 4
Effect of Enzyme Concentration
on Enzymatic Reaction Rate

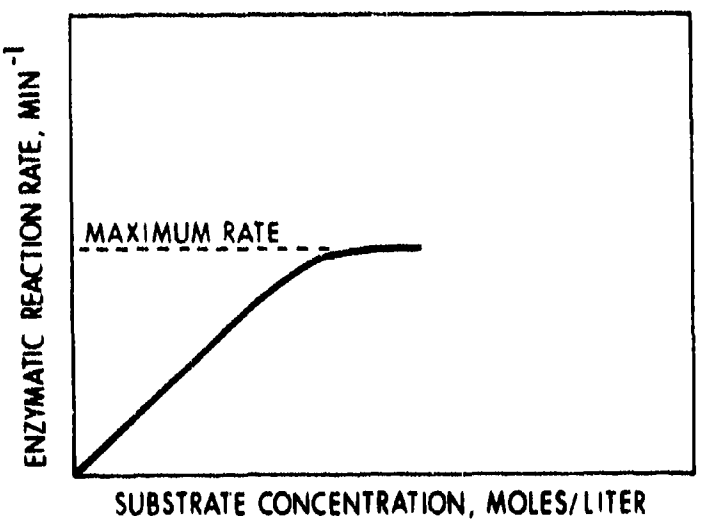

Figure 5
Effect of Substrate Concentration
on Enzymatic Reaction Rate

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